affinity despite drastic conformational changes. The results are important for understanding the fundamental principles and underlying forces that generate movement within molecular machines.

27-Subg
New Insight into Lipid-Protein Membrane Organization and its Functionality with Super-Resolution STED Microscopy
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Stimulated Emission Depletion (STED) far-field microscopy allows the study of living cells with nanoscale resolution, otherwise impeded by the limited spatial resolution of conventional microscopes. Besides the recording of images, the combination of STED with single-molecule sensitive spectroscopic tools such as Fluorescence Correlation Spectroscopy (FCS) discloses complex dynamical processes hidden to the conventional observations. For example, STED-FCS offers novel insights into important cellular processes, such as lipid-lipid, lipid-protein interactions or the formation of so-called ''lipid-rafts'' in the cellular plasma membrane, and their role in cellular functionality. Improved insights are realized by the implementation of gated detection or by recording STED-FCS data during scanning.

28-Subg
Proteins as Mechano-Chemical Signalling Switches
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Since the physical and biochemical properties of extracellular matrix provide critical cues to bacteria and cells, from mechanoregulated bacterial adhesion to angiogenesis, and finally to the differentiation of stem cells, it is of major importance to gain mechanistic insights into how mechanical stretching of extracellular matrix molecules can alter various cell functions. While investigating these three distinct physiological processes, common motifs are emerging how bacteria and cells take advantage of mechanical forces to regulate the function of proteins by stretching them out of their equilibrium structures. In this context, new assays and techniques were developed that allow probing how the stretching of proteins alters their structure-function relationships. Taken together, new insights into various underpinning mechanotransduction events are emerging how mechanical cues are translated into biochemical signals that ultimately regulate bacterial adhesion and various cellular processes.

Subgroup: Intrinsically Disordered Proteins

29-Subg
Structural Studies on the Activation and Substrate Binding of a Conditionally Disordered Acid-Activated Chaperone
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HdeA is a 9.7kDa chaperone that is inactive at neutral pH but becomes activated and disordered by shifts to low pH. The chaperone is required for bacterial pathogens to resist the acid-mediated protein aggregation that would otherwise occur in the human gut. We sought to identify which of HdeA's acid-titratable residues were the key players in its activation. Using constant pH molecular dynamics calculations and site-specific mutagenesis, we identified several residues involved in HdeA activation and have isolated variants that are destabilized and constitutively active at even neutral pH. These mutants help us to understand how pH-driven changes in HdeA flexibility drive activation.
One of the pressing problems in chaperone biology is how chaperones interact with a multitude of client proteins to facilitate their folding. HdeA's size and accessibility to NMR also makes it ideal for monitoring the structural changes that take place in this chaperone upon activation and upon client binding. Our predicted NMR structure of HdeA at neutral pH is a folded protein and very similar the crystal structures previously solved for the HdeA dimer. Upon shift to low pH however, HdeA simultaneously becomes active as a chaperone and acquires a largely disordered conformation. We have obtained structural information that gives us insights into HdeA's activation process and client binding. The use of small NMR accessible client proteins raises the exciting possibility of monitoring the structural changes that take place within the client upon interaction with the chaperone and in doing so gaining insight into the fundamental question of what chaperones do to their clients to facilitate folding.

30-Subg
Alpha-Synuclein, an Intrinsically Unstructured Protein. How Interesting Can It Be?
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N-terminal acetylation of alpha-synuclein (aS), a 140-residue protein implicated in the etiology of Parkinson's disease, is common in mammals. The impact of this modification on the protein's structure and dynamics in free solution and on its membrane binding properties has been evaluated by both NMR and CD spectroscopy. While in contrast to literature reports, no tetrameric form of acetylated aS could be isolated, N-terminal acetylation resulted in ca 15% transient population of alpha-helical structure for its first six residues. The 1H, 15N, and 13C chemical shifts for residues 13-140 remain unaffected by acetylation. Nevertheless, a substantial increase in affinity of aS for negatively charged lipid membranes is observed, likely to be of strong functional significance. A new method for residue-specific NMR probing of lipid binding is demonstrated for aS and assigns a new putative function to this enigmatic protein. Although free aS in the absence of lipids has backbone chemical shifts that are exceptionally close to random coil values, considerable positional variation for the distribution of its backbone torsion angles and the time scale of local reorientation can be deduced from its 3JHH and NOE data.

31-Subg
Intrinsic Protein Disorder in the Regulation of Large Molecular Machines
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We here report the molecular overlap of the linkage of three essential protein complexes that coordinate the formation of the mitotic spindle. These proteins are dynein, a large motor complex that moves machinery inside cells, and two of its regulators: a protein complex called dynactin, that is a dynein activator, and a protein called NudE whose depletion in mice produces a small brain and mental retardation. What is intriguing about the dynein/dynactin/NudE interplay is that dynactin and NudE bind to a common segment of dynein that is intrinsically disordered but with distinct binding modes. Elucidating details of these distinct modes explains how one regulator is selected over the other even when both are present in the same cellular compartment. Using NMR spectroscopy and isothermal titration calorimetry we show that intrinsic disorder in a specific segment of dynein intermediate chain promotes local modifications like phosphorylation and splicing, promotes rapid equilibration of ensemble components in solution samples of complexes with moderate to weak binding affinities, and creates a bi-segmental binding site such that residue-level modification in and near one segment have minimal effect on the structure of the second segment thereby aiding in segregating of functions between two consecutive segments. These results underscore the role of disorder in the versatility of dynein binding to different regulators, and have far reaching impact not only on our understanding of processes essential for formation and orientation of the spindle, but also offer a novel role for protein disorder in controlling cellular processes, and highlight the advantages of NMR spectroscopy in elucidating atomic level characterization of extremely complex dynamic cellular assemblies.

32-Subg
Probing the Polymeric Properties of Unfolded and Disordered Proteins with Single-Molecule Spectroscopy
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Single-molecule spectroscopy provides new opportunities for investigating the structure and dynamics of unfolded and intrinsically disordered proteins (IDPs). The combination of single-molecule Förster resonance energy transfer (FRET) with nanosecond correlation spectroscopy, microfluidic mixing, and related methods can be used to probe their distance distributions and reconfiguration dynamics on a wide range of time scales, and even in heterogeneous environments. In view of the large structural heterogeneity of these systems, a description in terms of polymer physical principles is often a useful way of conceptualizing their behavior. I will provide examples ranging from the influence of amino acid composition and temperature on the structure and dynamics of unfolded proteins and IDPs to the effects of crowding and molecular chaperones.

33-Subg
A Long Disordered Linker in Nuclear Transport of Membrane Proteins
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Nuclear Pore Complexes (NPCs) embedded in the nuclear envelope allow selective transport of macromolecules between the cytosol and nucleoplasm. Transport factors shuttle cargo though the NPCs by interacting with the disordered proteins that encode the binding sites for the transport factors, the